

Chloropid flies (Diptera, Chloropidae) associated with pitcher plants in North America (#22231)

1

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I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.

Chloropid flies (Diptera, Chloropidae) associated with pitcher plants in North America

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We review the taxonomy and ecology of Chloropidae (Diptera) associated with pitcher plants (Sarraceniaceae) in North America. *Tricimba wheeleri* **sp. n.** is described from the pitchers of *Sarracenia alata* Alph.Wood and *S. leucophylla* Raf. in the southeastern United States (Alabama, Mississippi). *Aphanotrigonum darlingtoniae* (Jones) associated with *Darlingtonia californica* Torr. in northern California is redescribed, including the first description of male genitalic characters. Published records of other species of *Tricimba* Lioy in pitcher plants in North America are considered accidental or facultative occurrences; published records of *Aphanotrigonum* Duda as pitcher plant associates in eastern North America are probably errors in identification.

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Abstract

We review the taxonomy and ecology of Chloropidae (Diptera) associated with pitcher plants (Sarraceniaceae) in North America. *Tricimba wheeleri* sp. n. is described from the pitchers of *Sarracenia alata* Alph. Wood and *S. leucophylla* Raf. in the southeastern United States (Alabama, Mississippi). *Aphanotrigonum darlingtoniae* (Jones) associated with *Darlingtonia californica* Torr. in northern California is redescribed, including the first description of male genitalic characters. Published records of other species of *Tricimba* Lioy in pitcher plants in North America are considered accidental or facultative occurrences; published records of *Aphanotrigonum* Duda as pitcher plant associates in eastern North America are probably errors in identification.

Keywords: Oscinellinae, *Tricimba*, *Aphanotrigonum*, Nearctic, taxonomy, ecology

Introduction

There are two very different ways in which insects can associate with pitcher plants (Sarraceniaceae): they can die in the modified, pitcher-shaped leaves and become a source of nutrients for the plant; or they can live in the pitchers and become part of a fascinating ecosystem. Several families of Diptera in North America include pitcher plant associated species: ten species of Sarcophagidae (Dahlem and Naczi 2006) and one or two species each of Culicidae, Chironomidae, Sciaridae, and Chloropidae (Jones 1916, 1920, Szerlip 1975, Folkerts 1999, Dahlem and Naczi 2006).

Jones (1916) described a new species of Chloropidae, *Botanobia darlingtoniae* Jones (transferred to *Aphanotrigonum* Duda by Sabrosky (1965)) from specimens reared from pitchers of *Darlingtonia californica* Torr. in California, but that fly has not been documented in association with pitcher plants since its original description. There are records of a second, undescribed species of *Aphanotrigonum* Duda from multiple species of *Sarracenia* L. in eastern North America (Folkerts 1999). However, based on examination of specimens collected from *Sarracenia* spp. in eastern North America, the eastern chloropids are not congeneric with *A. darlingtoniae* but actually belong to *Tricimba* Lioy, an externally similar, but distantly related, genus.

In this paper, we revise the two species of Chloropidae known to be associated with pitcher plants in North America, with a redescription of *A. darlingtoniae* from the western United States and a description of *Tricimba wheeleri* **sp. n.** from the southeastern United States. Adults of *T.*

wheeleri have unusual modifications of the tarsi not known in other species of *Tricimba*, possibly associated with locomotion by adult flies inside pitchers of their host plants.

Methods

Specimens studied are deposited in the following collections: Lyman Entomological Museum, McGill University, Ste-Anne-de-Bellevue, Quebec, Canada (LEMQ); Personal collection of Robert F.C. Naczi (RFCN); United States National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM).

Two specimens of *A. darlingtoniae* from LEMQ were submitted to the Canadian Centre for DNA Barcoding (University of Guelph, Guelph, Ontario, Canada) for sequencing of the 658 bp DNA barcode region of the mitochondrial gene CO1.

Male genitalia were prepared by removing the abdomen of pinned specimens and clearing them in 85% lactic acid heated in a microwave oven for 1-2 periods of 10 seconds, separated by a 1 minute cooling period.

Morphological terms follow Cumming and Wood (2009).

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that

68 Code from the electronic edition alone. This published work and the nomenclatural acts it
 69 contains have been registered in ZooBank, the online registration system for the ICZN. The
 70 ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed
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 73 B00951574F39. The online version of this work is archived and available from the following
 74 digital repositories: PeerJ, PubMed Central and CLOCKSS.

Results and Discussion

Aphanotrigonum darlingtoniae (Jones)

Botanobia darlingtoniae Jones, 1916: 389. Type locality: Mount Eddy, near Sisson, Siskiyou County, California (see Comments).

Aphanotrigonum darlingtoniae: Sabrosky, 1965: 785.

Type material. Holotype **M** labelled: “Sisson, Cal./Bred. F. M. Jones./VIII-5-1915”; “m# TYPE”; “Type No./20318/U.S.N.M.”; “Oscinella/darlingtoniae/Type.) Jones” (USNM). Allotype F: same data as holotype except “VIII-15-1915”; “f# TYPE”; “Allotype No./20318/U.S.N.M.” (USNM). Paratype **f#**: same except: “VII-29-1916” [probably error for 1915]; ‘Botanobia/darlingtoniae/Type] Jones”; red square blank label] (USNM) (See Remarks). Other material examined. USA: CA: Siskiyou Co. 6km NW Mt Eddy, Forest Road 17 (41.3573°, -122.5409°), 2060m, sweep wet *Darlingtonia* meadow, 08.vi.2009. J. Mlynarek (5M, 6F, LEMQ), same data except T.A. Wheeler (3M, 1F, LEMQ).

Diagnosis. (Figs. 1-2) *Aphanotrigonum darlingtoniae* can be distinguished from the other described Nearctic species of the genus (*A. scabrum* (Aldrich), *A. trilineatum* **Author**) by the combination of: dark bristles on gena, three brown stripes on scutum in the shape of a lyre, sides of scutellum also brown (uniformly gray in paratypes of *scabrum* from Treesbank), interfrontal setulae are darker, more contrasting with frons in *darlingtoniae* and distinction between gray triangle and yellow anterior part of frons seems less clear in *darlingtoniae* than in *scabrum*.

99

100 Description (Fig 1). Total length 2.3–3.1 mm . Overall colour black, frontal triangle brown-
 101 black, pollinose, wide, 2/3 length of frons; ocellar tubercle black, pollinose; frons yellow to
 102 brown, wider than long; cephalic setae dark, strong but short, frontal-orbital bristles short, black
 103 and convergent, two rows of interfrontal setulae inside ~~the~~ margin of frontal triangle; gena wide,
 104 anterodorsally yellow, ventrally black, 0.3–0.4 times eye height; eye bare; postgena black,
 105 pubescent and 1/5 width of eye; face yellow to brown; scape and pedicel yellow, first
 106 flagellomere oval, 1.25–1.35 higher than long, first segment of arista black, rest of arista black,
 107 slender and 2–2.5 times ~~the~~ width of ~~the~~ first flagellomere; palpus yellow, proboscis brown,
 108 clypeus black and shiny.

109

110 Scutum black with three brown dorsal lines, ~~pollinose giving a grey appearance,~~ 1.2–1.4 times
 111 wider than long, pronotum black, shiny, thin; scutellum black, pollinose, 1.4–1.5 times as wide
 112 as long, apical scutellar bristles black and stronger than surrounding setae, on very small
 113 tubercles; thoracic ~~pleurites~~ pollinose; dorsal margin of anepimeron and lateral region of
 114 postscutellum pollinose. femur black, **tarsus** yellow with thick black band, metatarsi yellow;
 115 femoral organ absent; tibial organ present, black, 0.3 length of tibia. Ratio of costal sectors
 116 C1:C2:C3:C4 on wing 1:1.3:1:0.5.

117

118 Abdomen black, pollinose, longer than thorax; syntergite 1+2 same length as remaining tergites.

119

120 Male postabdomen (Fig 2): small; epandrium in lateral view 1.4 times higher than wide, in
 121 posterior view 1.5 times wider than high, setae sparse covering posterior portion of epandrium;

surstylus 0.75 times as high as epandrium, parallel sided with ~~a~~-rounded tip, setae short and sparse; cercus square **posteriorly**, triangular **laterally**, separated by ~~a~~-small evenly rounded arc, bristles short with one long bristle on the posteromedial edge; distiphallus not well sclerotized.

Molecular sequence data. DNA barcodes (658 bp of CO1) for two LEMQ specimens of *A. darlingtoniae* are available on the Barcode of Life Database (boldsystems.org), (BOLD accession numbers: CCDB-21328-A12/LYMAA1247-14; CCDB-21328-B01/LYMAA1248-14).

Remarks. **Jones** recorded the type locality as “Mount Eddy, near Sisson, Siskiyou County, California”. Sisson is the ~~previous~~ name for the town of Mount Shasta. There are several populations of *Darlingtonia californica* in the area surrounding Mount Eddy, west of Mount Shasta, so it is impossible to determine precisely the type locality. The second female **type** specimen is not labelled as a type but is assumed to be a paratype because the emergence data matches that given in the original publication. In addition, the determination label is in Jones’ handwriting.

Our 2009 collections occurred early in the season (Fig 3). *Darlingtonia* pitchers were small and pale green, in contrast to the previous year’s dark pitchers. There were still patches of snow in the open forest near the collecting site and other herbaceous plants in the vicinity were early in their annual development. Sampling was non-destructive; we swept just above the substrate adjacent to the developing pitchers and over the debris of the previous year’s growth. Jones (1916) based his description of *A. darlingtoniae* on adult specimens reared from pitchers of *D. californica*, but our collecting suggests that adults can live outside pitchers and, given the small size of new pitchers, **the adult flies may overwinter** in the substrate outside the pitchers.

Our collected *A. darlingtoniae* from California clusters in same BIN as *Aphanotrigonum trilineatum* from Alberta, British Columbia, and New Brunswick. We morphologically compared the two species to confirm the validity of *A. darlingtoniae*. We also dissected and compared male genitalia. Even though they share the same BIN and molecular barcode, *Aphanotrigonum darlingtoniae* is morphologically distinct from *A. trilineatum* supporting the validity of both species.

***Tricimba wheeleri* Mlynarek new species**

LSID: urn:lsid:zoobank.org:act:C9CC48AD-AE7A-49DB-B849-2C64B6C5E812

Type locality. USA: Alabama: Mobile County, 5mi W of Citronelle.

Type material. Holotype M: USA: Alabama: Mobile County, 5mi W of Citronelle, 31.08°N 88.3°W 2.viii.1994, R.F.C. Naczi, Ex. *Sarracenia leucophylla* (LEMQ). Paratypes: 24 M, 31 F: same as holotype; 1 F: Theodore, 21.vi.1915, F.M. Jones (USNM); Mississippi: 14 M, 22 F: Stone County, 9mi E of Wiggins, 30.8°N 88.9°W 1.viii.1994, R.F.C. Naczi, Ex *Sarracenia alata*. (LEMQ).

Other material (not examined). USA. Alabama: Baldwin Co., 0.9 mi NNE of Perdido, 31.0206°N, 87.6234°W, 28 April 1988; R. F. C. Naczi, As adults from inside upper portions of *Sarracenia leucophylla* pitchers. (2M 3F, RFCN); ~~USA~~. Florida: Okaloosa Co., 3 mi S of Crestview, 30.6990°N, 86.5745°W, 19 Aug. 1984; R. F. C. Naczi, As adults from inside upper portions of *Sarracenia leucophylla* pitchers. (3M, RFCN)

Diagnosis (Figs 4-5). *Tricimba wheeleri* can be distinguished from the other described Nearctic species of the genus by the combination of: shallowly incised scutal lines, and heavily microtomentose gray scutum, **expanded distal tarsal segments**.

Description (Fig 4). Total length 1.7–2.7 mm. Overall colour brown to black, frontal triangle black, pollinose, 0.4–0.5 times ~~the~~ length frons; ocellar tubercle black, pollinose; frons yellow anteriorly, darkening posteriorly until black, size 1.2–1.3 times longer than wide; cephalic setae slender and pale, frontal-orbital bristles even, interfrontal setulae on margin of frontal triangle; gena yellow, pollinose, 0.2–0.3 times eye height; eye bare; postgena black, pollinose and thin; face brown, as high as wide; scape and pedicel yellow, first flagellomere yellow proximoventrally darkening distodorsally, negligibly higher than wide, first segment of arista yellow to brown, rest of arista slender, brown, sparse hairy and twice as long as first antennal segment; palpus and proboscis yellow, clypeus brown.

Scutum black, pollinose, with three weakly incised dorsal lines along the dorsocentral ~~and~~ **medial** lines, covered in fine yellow hairs, 1–1.2 times longer than wide, pronotum black and shiny; postpronotum brown to black; scutellum **black**, round, 1.6–1.8 times as wide as long, apical scutellar bristles black ~~and a lot~~ longer than surrounding setae, on small tubercles; thoracic ~~pleurites~~ brown to black, pollinose; dorsal margin of anepimeron and lateral region of postscutellum pollinose. Legs brown, joints sometimes paler; femoral organ absent; tibial organ present, yellow to black, large, 0.3 length of tibia. Ratio of costal sectors C1:C2:C3:C4 on wing 1:0.95:0.6:0.5.

Abdomen black, pollinose, longer than thorax; syntergite 1+2 same length as remaining tergites.

Male postabdomen (Fig 5): large; epandrium in lateral view as high as wide, in posterior view 1.4 times as wide as high, setae dense covering posterior end of epandrium; surstylus 0.8 times the height of the epandrium, setae short and sparsely covering entire surstylus; cercus large, parallel sided and rounded, separated by a small evenly rounded arc, bristles short covering entire cercus; distiphallus small and poorly sclerotized.

Etymology. The species name is a genitive patronym in honour of Terry A. Wheeler, the co-author of this manuscript who passed away during the final stages of this project and contributed much to our knowledge of the Chloropidae. He always said that “this is probably the last species of insect that is associated with North American pitcher plants that needs to be described, how fitting it should be a chloropid”.

Remarks. In addition to collecting a single paratype, F.M. Jones made notes on additional specimens from Alabama, North Carolina, and South Carolina apparently belonging to this species. Unpublished sketches in the F.M. Jones archives, deposited in the Peabody Museum of Natural History, Yale University, New Haven, CT, USA (<http://harvardforest.fas.harvard.edu/ellison/current-research/Frank-Morton-Jones>), of specimens collected from *Sarracenia drummondii* (= *S. leucophylla*), *S. flava*, *S. minor* and *S. rubra* illustrate the expanded distal tarsal segments, long tarsal claws, and expanded pulvilli.

The type specimens from *Sarracenia leucophylla* and *S. alata* were collected as adults from inside the upper portion of the pitchers (RFC Naczi, pers. comm.)

The tarsal modifications are not present in other Nearctic *Tricimba* species and may be an adaptation for walking on the surface of the debris inside pitchers (Fig 6).

Folkerts (1999) recorded an undescribed species of “*Aphanotrigonum*” from *Sarracenia* species in the eastern United States and that record has been repeated by others (e.g., Dahlem and Naczi 2006). Although we have not seen vouchers of the specimens referred to by Folkerts, that record likely refers to *Tricimba wheeleri*. Folkerts (1999) referred to “*Aphanotrigonum macfarlanei*” from *Sarracenia* species in the eastern United States as the sister species to *Aphanotrigonum darlingtoniae*; however, *A. macfarlanei* is not a valid species and this record appears to be an error based on the conflation of *Aphanotrigonum* with *Bradysia macfarlanei* (Jones) (Diptera: Sciaridae), another known inhabitant of *Sarracenia* pitchers in the southeastern United States. There is, obviously, no evidence of a sister group relationship between the western and eastern pitcher plant Chloropidae, given that they belong to different genera.

***Tricimba* sp.**

Material examined. USA: California: Del Norte Co., Gasquet, 31.iii.1986, emerged 14.iv.1986, D.W. Nielsen, ex larvae coll. in pitchers of *Darlingtonia californica* (1M, USNM); same data except emerged 23.iv.1986 (1F, USNM).

Remarks. Two specimens identified as an undescribed species of *Nartshukiella* (= *Tricimba*) nr. *melancholica* (Becker) were reared from a single *Darlingtonia californica* pitcher (of a total of

235 124 pitchers sampled) in Del Norte County, California (~~Nielsen, 1990~~). At the time of collection,
 236 flies were sent to C.W. Sabrosky (USNM) for identification and are still deposited in USNM.
 237 The specimens are a typical *Tricimba melancholica* group species. The tarsi are not modified as
 238 in *T. wheeleri*. Given that only two specimens were reared in the course of a large-scale study of
 239 *Darlingtonia* insect associates, this may have been an opportunistic colonization of a single
 240 pitcher plant by a generalist saprophagous species. The association should be considered at most
 241 facultative and likely accidental.

242 Conclusion

243 There are two species, *Aphanotrigonum darlingtoniae* and *Tricimba wheeleri* (described here),
 244 which live in the pitchers of North American pitcher plants and have become part of that
 245 fascinating ecosystem. *Aphanotrigonum darlingtoniae* is associated with *Darlingtonia*
 246 *californica* in Northern California. *Tricimba wheeleri* is associated with *Sarracenia* species in
 247 southeastern North America. Another species of *Tricimba* that ~~have been~~ rarely reared from
 248 *Darlingtonia* should be considered facultative.

249

250 **Acknowledgments**

251 Fieldwork in California was facilitated by Steve Gaimari and Peter Kerr (California Department
 252 of Food and Agriculture). Rob Naczi (New York Botanical Garden) provided type specimens
 253 and ecological information on *Tricimba wheeleri*. Aaron Ellison (Harvard Forest) connected us
 254 with the F. M. Jones archives and Steve Marshall (University of Guelph) alerted us to the Szerlip
 255 (1975) paper. Norm Woodley (USNM) arranged access to the USNM collection. Staff at the
 256 Biodiversity Institute of Ontario, especially Valérie Lévesque-Beaudin, collaborated on the DNA
 257 barcoding of *A. darlingtoniae*. Stephen heard made helpful comments on the manuscript.

258

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Figure captions

Figure 1. *Aphanotrigonum darlingtoniae* , male habitus. A. Dorsal. B. Lateral. Scale bar = 1mm

Figures 2. *Aphanotrigonum darlingtoniae*, male genitalia. A. Lateral. B. Posterior. C. Ventral.

Abbreviations: cer, cerci; epd, epandrium; hyp, hypandrium; phap, phallapodeme; sur, surstylus.

Scale bar = 0.1mm

Figure 3. 2009 collecting locality of *Aphanotrigonum darlingtoniae*. Mt Eddy, Siskiyou County, California.

Figures 4. *Tricimba wheeleri*, male habitus. A. Dorsal. B. Lateral. Scale bar = 1mm

Figures 5. *Tricimba wheeleri*, male genitalia. A. Lateral. B. Posterior. C. Ventral. Scale bar = 0.1mm

Figures 6. A. *Tricimba wheeleri*, male fore tarsus. B. *Tricimba melancholica*, male fore tarsus.

Scale bars = 0.1mm

Figure 1

Aphanotrigonum darlingtoniae, male habitus.

A. Dorsal. B. Lateral. Scale bar = 1mm



Figure 2

Aphanotrigonum darlingtoniae, male genitalia.

A. Lateral. B. Posterior. C. Ventral. Abbreviations: cer, cerci; epd, epandrium; hyp, hypandrium; phap, phallapodeme; sur, surstylus. Scale bar = 0.1mm

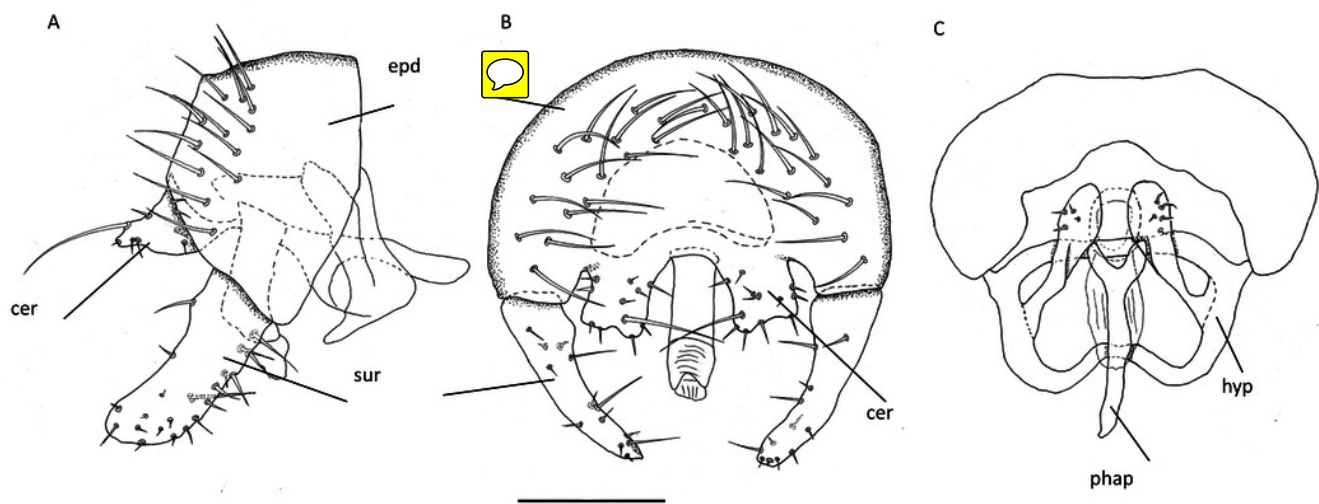


Figure 3

2009 collecting locality of *Aphanotrigonum darlingtoniae*. Mt Eddy, Siskiyou County, California.



Figure 4

Tricimba wheeleri, male habitus.

A. Dorsal. B. Lateral. Scale bar = 1mm

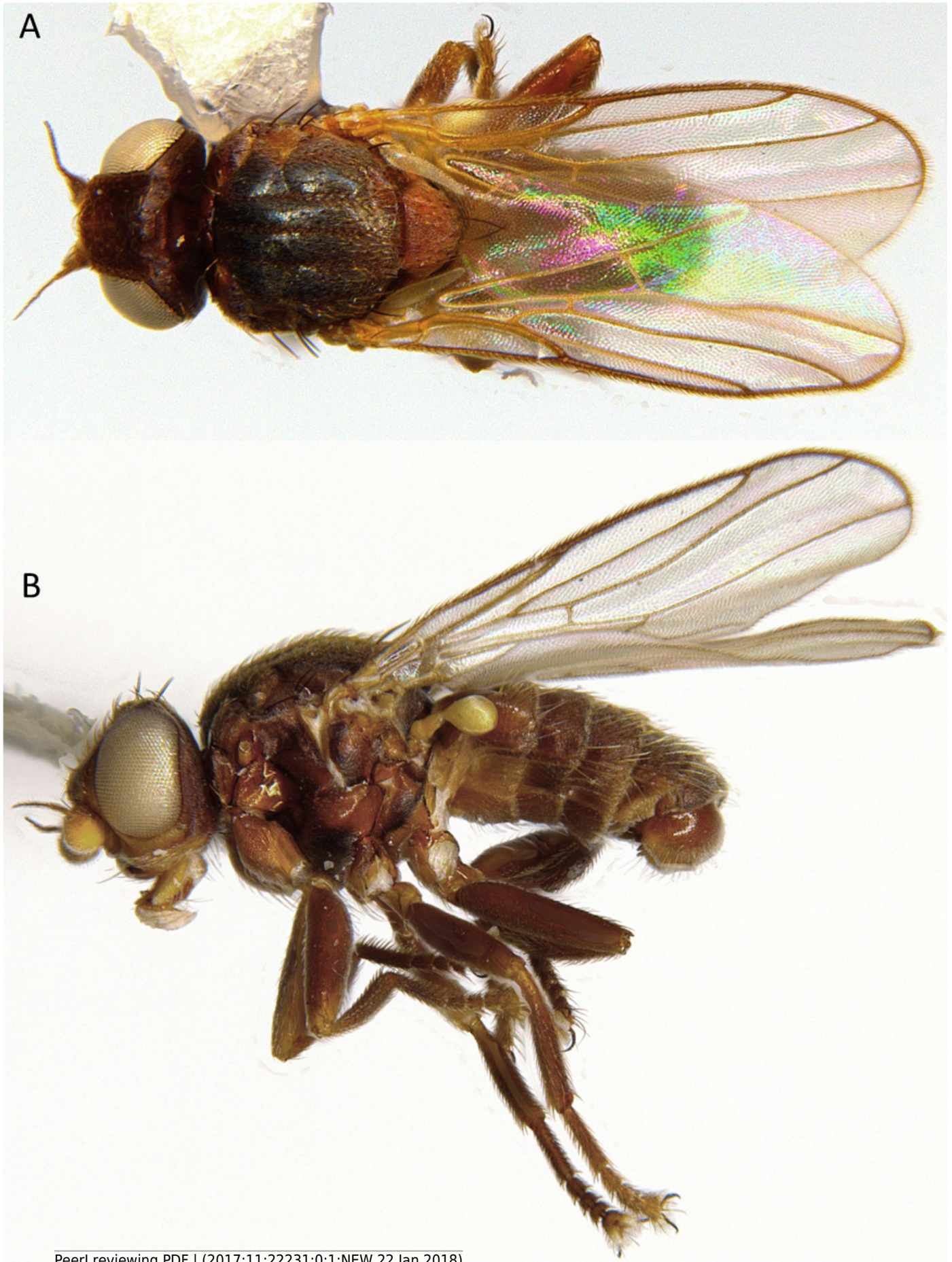


Figure 5

Tricimba wheeleri, male genitalia.

A. Lateral. B. Posterior. C. Ventral. Scale bar = 0.1mm

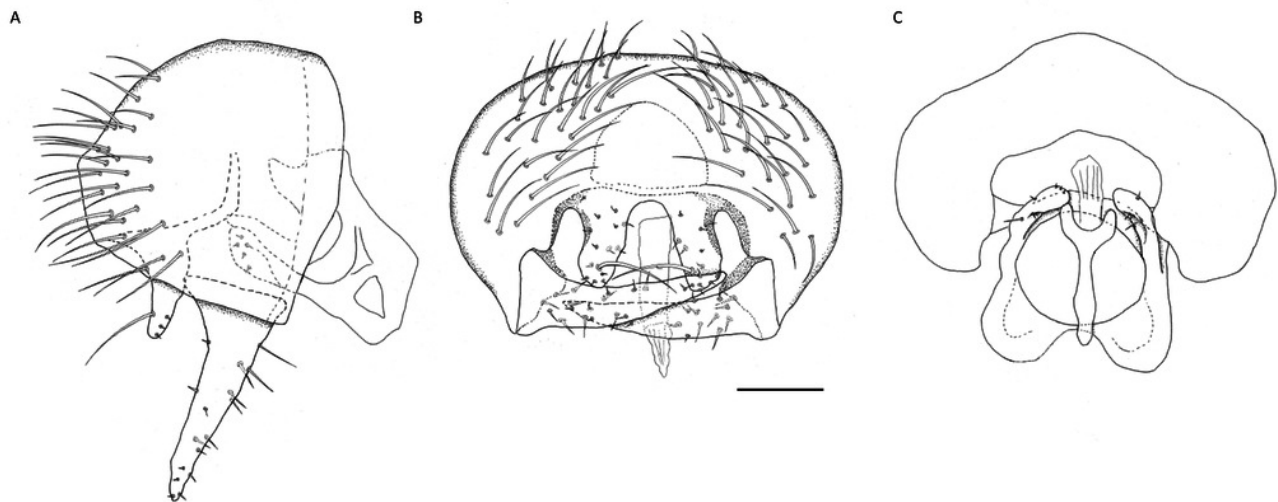


Figure 6

A. *Tricimba wheeleri*, male fore tarsus. B. *Tricimba melancholica*, male fore tarsus.

Scale bars = 0.1mm

